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Mini Review

Oxidative post-translational modifications and their involvement in the pathogenesis of autoimmune diseases

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ABSTRACT

Tissue inflammation results in the production of numerous reactive oxygen, nitrogen and chlorine species, in addition to the products of lipid and sugar oxidation. Some of these products are capable of chemically modifying amino acids. This in turn results in changes to the structure and function of proteins. Increasing evidence demonstrates that such oxidative post-translational modifications result in the generation of neo-epitopes capable of eliciting both innate and adaptive immune responses. In this paper, we focus on how free radicals and related chemical species generated in inflammatory environments modulate the antigenicity of self-proteins, resulting in immune responses which involve the generation of autoantibodies against key autoantigens in autoimmune diseases. As examples, we will focus on Ro-60 and C1q in systemic lupus erythematosus, along with type-II collagen in rheumatoid arthritis. This review also covers some of the emerging literature which demonstrates that neo-epitopes generated by oxidation are conserved, as exemplified by the evolutionarily conserved pathogen-associated molecular patterns (PAMPs). We discuss how these observations relate to the pathogenesis of both human autoimmune diseases and inflammatory disease, such as atherosclerosis. The potential for these neo-epitopes and the immune responses against them to act as biomarkers or therapeutic targets is also discussed.

Introduction

The breakdown of immune tolerance – and subsequent production of antibodies against host macromolecules – is a complex process involving genetic and environmental factors, as well as B and T cell dysregulation. However, a key feature in the initiation of many autoimmune diseases is the post-translational modification of antigens, which results in the recognition of host proteins as 'non-self' or 'dangerous' and thus the initiation of an adaptive immune response. Oxidative stress is a key feature of many inflammatory autoimmune diseases and results in an excess of reactive chemical species that are able to post-translationally modify proteins, potentially forming neo-epitopes [1–8]. These neo-epitopes may directly elicit an adaptive immune response or influence well-accepted immunological phenomena such as molecular mimicry (a host antigen being 'seen' as a 'non-self' protein), exposure of cryptic epitopes (exposure of amino acid sequences after changes in the three-dimensional structure of a protein), epitope spreading (spreading of antigenicity from a given epitope to other parts of the protein or other proteins) and the coupling of an autoantigen to an exogenous antigen [9–11]. All of the above results in breakdown of immune tolerance.

Neo-epitopes are able act as pathogen- or danger-associated molecular patterns (PAMPs/DAMPs) and are sensed by the immune system via pattern recognition receptors (PRRs) such as scavenger receptors, receptor of advanced glycation endproducts (RAGE), Toll-like receptor 4 (TLR-4) or natural (IgM isotype) antibodies [12–14]. This subject has been extensively reviewed in the context of atherosclerosis [14]. Increased generation of neo-epitopes/PAMPs/DAMPs may therefore serve as a mechanism for increased uptake and presentation of autoantigens to the immune system. Prolonged/repeated exposure of antigens to the immune system in this way is a known route for the initiation of class switching in the adaptive immune response [15–18].

Oxidative modifications are not the only post-translational modifications (PTMs) that may initiate autoantigenicity. Enzymatic PTMs are well-documented. For example, the ability of granzyme B to proteolytically cleave a particular protein (thereby generating antigenic cleavage products) is a predictor of autoantigenicity [19]. Another well-known example of enzymatic PTMs is the citrullination of arginine by peptidyl arginine deiminase (PAD), forming citrulline. This PTM generates epitopes that provide a highly sensitive and specific

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assay for the diagnosis of rheumatoid arthritis (RA; discussed in detail below) [20,21]. Other modifications include the methylation of spliceosomal proteins in systemic lupus erythematosus (SLE) and the deamidation of the coeliac autoantigen, gliadin, by transglutaminase [22,23]. This subject has previously been reviewed elsewhere [24].

Oxidative modifications of DNA, proteins and lipids all increase their antigenicity with relevance to both autoimmune and other diseases with an inflammatory component. Whilst there is no doubt that in autoimmune diseases such as RA, SLE and type-1 diabetes mellitus (T1D) the generation IgG isotype autoantibodies plays a direct role in pathogenesis, there is an increasing appreciation of the potential role of 'natural' IgM isotype autoantibodies in the early stages of autoimmune triggering. Indeed, it is estimated that 30% of all natural IgM antibodies, secreted by a subset of B cells (B1 cells), target oxidation-specific epitopes and may act to neutralise both pathogens and neo-epitopes [25].

The methods for the measurement of antibodies to oxidatively modified proteins in autoimmune diseases, and subsequent assessment of their clinical utility, have been described elsewhere [26,27]. This review will detail examples of how oxidation, nitration, lipid peroxidation and advanced glycation end-product formation influence the breakdown of tolerance towards proteins in autoimmune diseases, with SLE and RA as primary exemplars.

Modification of proteins by free radicals and other reactive species

Reactive oxygen, nitrogen and chlorine species

A number of cellular processes and enzymes are capable of generating reactive oxygen, nitrogen and chlorine species *in vivo*, in response to a wide variety of stimuli during homeostasis and during inflammation. The enzymatic generation of free radicals and other reactive species is largely governed by enzyme expression/activity, subcellular localisation and the presence of cofactors, whereas non-enzymatic reactive species generation is usually stochastic in nature. During inflammation, phagocyte NADPH oxidase (NOX2), inducible nitric oxide synthase (iNOS), and myeloperoxidase (MPO) all act as major sources of reactive oxygen, nitrogen and halide species capable of modifying proteins or generating secondary electrophilic species such as lipid peroxidation products and products of glucose oxidation. A wide variety of amino acids are susceptible to modification by these reactive species. The types of modified amino acids are governed by a number of factors including the rate constant for the reaction between the oxidant and the given amino acid residue, the solvent exposure of the target residue, steric hindrance of the target residue, the pKa of the residue and the identity of the surrounding residues. In relation to antibody production, solvent-exposed neo-epitopes are more likely to initiate an immune response.

A range of reactive oxygen, nitrogen and chlorine species are capable of modifying proteins, including the hydroxyl radical ($^{\bullet}OH$), the carbonate radical ($CO_3^{\bullet-}$), hypochlorite (OCl^-) and products formed from reactions involving peroxynitrite ($ONOO^-$). Cysteine residues on proteins are readily oxidised due the free thiol (–SH) group. This free thiol may be oxidised to form either an inter- or intra-molecular disulfide bond with another free thiol (–S–S–). In addition, cysteine may be oxidised to cysteine sulfenic, sulfinic and sulfonic acid derivatives (Fig. 1a). This occurs in the presence of oxidants such as $^{\bullet}OH$ and is used by antioxidant systems such as the peroxiredoxin/thioredoxin system [28,29]. Importantly, cysteine is a target of S-nitrosation. Although this modification is important for cellular signalling, S-nitrosothiols are unlikely to represent neo-epitopes capable of eliciting an immune response, given the labile nature of the S–NO bond (Fig. 1a). Similarly, methionine can be oxidised to methionine sulfoxide and further to methionine sulfone (Fig. 1b) [30]. Other oxidative modifications to amino acids include tryptophan oxidation to hydroxytryptophan and further to kynurenine (Fig. 1c), and tyrosine oxidation to 3,4-dihydroxyphenylalenine, or inter- or intra-molecular di-tyrosine cross-linking via the formation of tyrosyl radicals (Fig. 1d) [31,32].

Nitration of tyrosine and tryptophan residues in inflamed tissues has been extensively reported in the literature and has been demonstrated to modulate the structure and function of proteins in many disease states. The relatively stable nature of the modification indicates it may be a good candidate for neo-antigen formation (Fig. 1d) [33,34]. Chlorination of proteins, mediated by HOCl production by MPO is another modification that can occur on proteins, altering their function. Chlorination has been most widely reported on tyrosine residues, forming 3-chlorotyrosine. Chloramines on lysine and histidine residues constitute important intermediates in stable end products of chlorination (Fig. 1d) [35,36]. It is this chlorination of proteins and DNA that is responsible for the effects of MPO-derived HOCl during the respiratory burst [37].

Lipid peroxidation products

Oxidation of unsaturated double bonds in lipids, by species such as $^{\bullet}OH$ can result in the formation of lipid peroxyl radicals ($RO_2^{\bullet}$). These radicals can then act on other unsaturated lipids to propagate lipid peroxidation. This lipid peroxidation can disrupt cell membranes leading to necrosis or the induction of apoptosis of cells, through modification of DNA or proteins. Two major reactive species produced by lipid peroxidation at sites of inflammation are malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) (Fig. 2a), both of which are capable of reacting with a number of (nucleophilic) amino acid residues in proteins, including lysine, arginine, methionine, tyrosine and histidine, primarily through 1,4 Michael addition reactions [38,39].

Products of glucose oxidation

The reactive electrophiles, glyoxal and methylglyoxal, are formed from the oxidation of glucose, a process that is increased under oxidative stress. The reaction of glyoxal and methylglyoxal with proteins results in the formation of AGEs such as pentosidine, which are associated with a number of inflammatory pathologies (Fig. 2b) [40,41]. These AGEs are formed through the Maillard reaction between reactive carbonyls and basic amino acid side chains such as lysine and arginine. AGEs have been implicated in a number of diseases. For example, serum levels of pentosidine are increased in both RA and SLE patients [42].

Systemic lupus erythematosus

Autoantigen reservoirs within uncleared apoptotic cells in SLE patients

SLE is a prototypic systemic autoimmune disease affecting multiple organs of the body. The clinical features of SLE are primarily a result of inflammation mediated by autoantibodies against host macromolecules, such as DNA and proteins. Autoantibodies form immune complexes with host molecules. These immune complexes can be deposited in the glomeruli of the kidney and in other organs causing inflammation.

Whilst the reason for the breadth of both cellular and peripheral antigens recognised in SLE patients is unknown, Casciola-Rosen et al. [43] demonstrated that a large number of lupus autoantigens are expressed on the surface of apoptotic cells and clustered in close proximity to oxidant-generating sites such as the endoplasmic reticulum. Later, it was demonstrated that enzymatic cleavage of a given protein by the serine protease, granzyme B, was a predictor of autoantigenicity and further that levels of granzyme B secreting CD8+ T-cells correlated with disease activity in SLE patients [19,44]. This observation is supported by the observed accumulation of apoptotic cells in the peripheral blood of SLE patients, indicating impairment

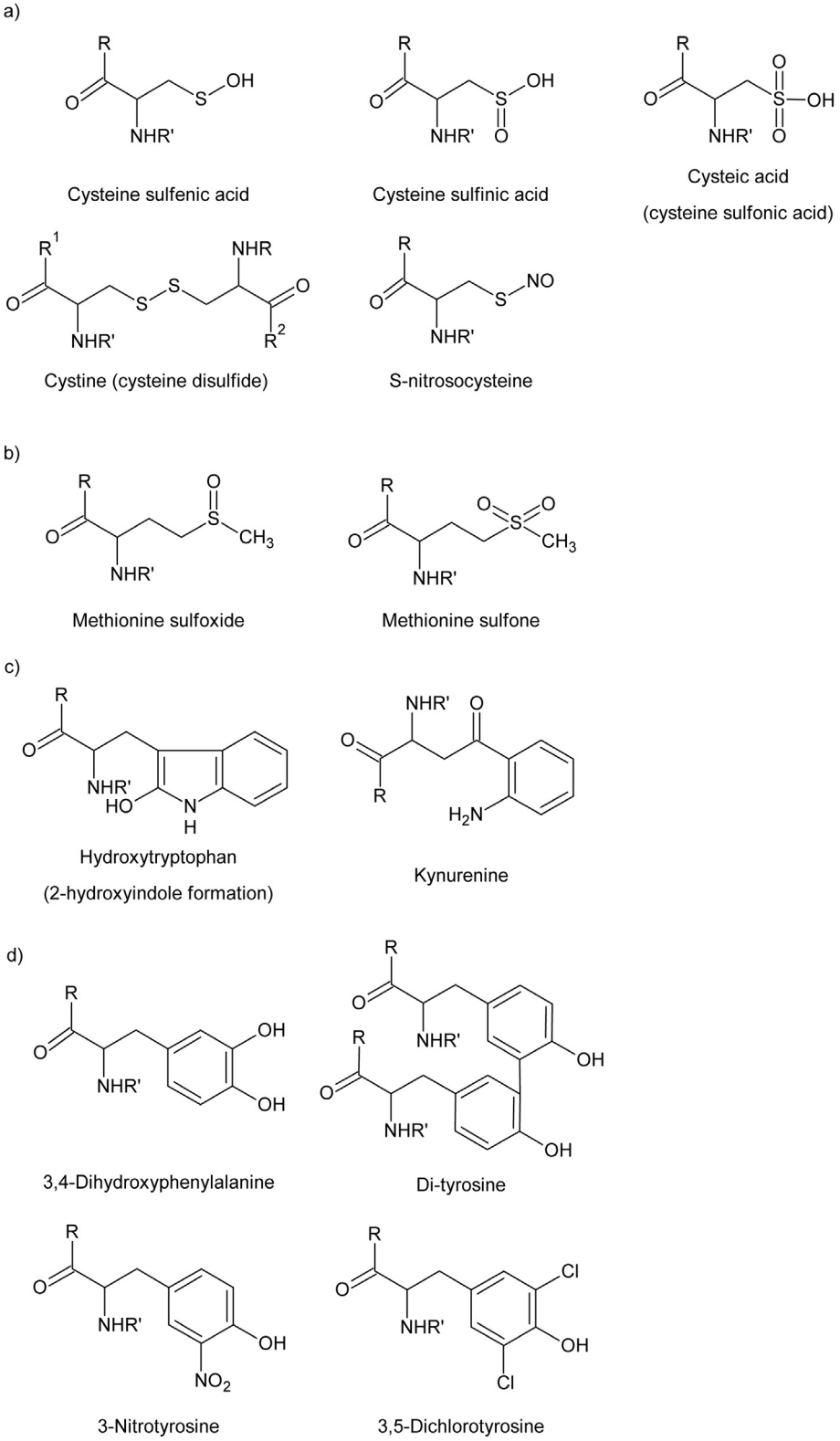

Fig. 1. Structures of amino acids after post-translational modifications by reactive oxygen, nitrogen and chlorine species. Structures of some of the post-translationally modified forms of (a) cysteine residues, (b) methionine residues, (c) tryptophan residues and (d) tyrosine residues.

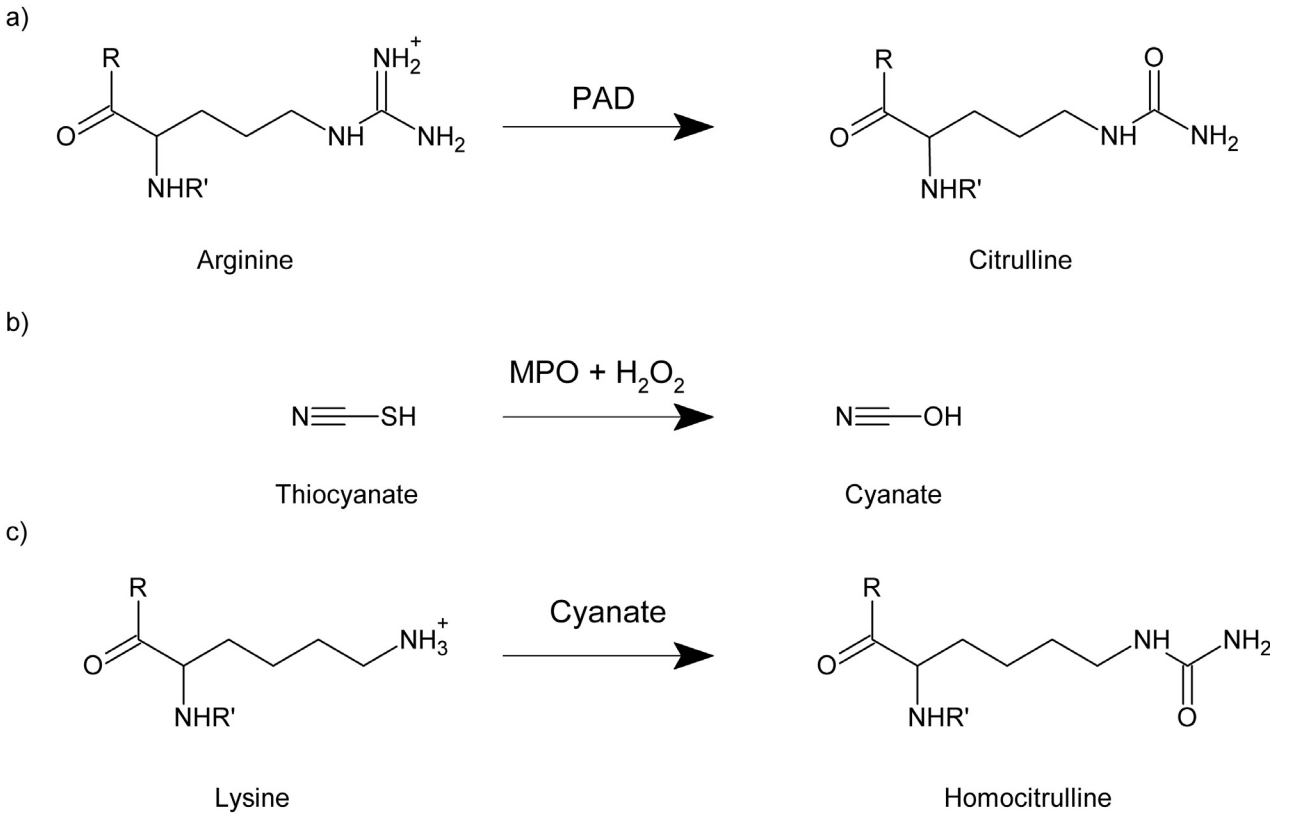

Fig. 2. Structures of some lipid and glucose oxidation products, and examples of the products formed by reactions of lipid/glucose oxidation products with amino acids within proteins – thereby generating post-translational modifications. Structures of (a) lipid peroxidation products (b) a product of lysine modification by MDA, (c) structures of the products of glucose oxidation, glyoxal and methylglyoxal, and (d) the AGEs carboxymethyllysine and pentosidine.

in the clearance of these apoptotic cells (impairment of efferocytosis) and the consequent presence of a reservoir of autoantigens associated with apoptotic cells. The uncleared apoptotic cells undergo secondary necrosis, a loss of membrane integrity and the spillage of intracellular proteins into the extracellular space [45,46]. Thus, in SLE patients there is a vicious cycle of oxidant generation and necrotic cell formation that stimulates the autoimmune response. It is therefore interesting to note that many of the same molecules which are responsible for the uptake of apoptotic cells also act as receptors for oxidised autoantigens e.g. CD36, RAGE and C-reactive protein [47,48].

Neo-epitope formation and epitope spreading in nuclear antigens

Oxidative stress is prevalent in SLE patients, as evidenced by an increase in oxidatively modified amino acids such as increased methionine sulfoxide, 3-nitrotyrosine and protein carbonyls, and decreased protein thiols. Indeed, levels of a number of these oxidatively modified amino acids correlated with disease activity [6,7,49]. Similarly, SLE patients have raised serum levels of MDA- and 4-HNE-modified proteins as well as AGEs [50,51]. In addition, antibody titres against MDA- and 4-HNE-modified ovalbumin were found to be raised in SLE patients and also correlated with disease activity. Modification of bovine serum albumin with polyunsaturated fatty acids resulted in cross-reactivity with sera from MRL-*lpr* mice, with 4-oxo-2-nonenal modification being identified as the main antigenic determinant [52]. However, a subset of anti-dsDNA antibodies from these mice also bound 4-oxo-2-nonenal-modified bovine serum albumin, indicating cross-reactivity between dsDNA and oxidatively modified antigens [52].

Approximately 90% of SLE patients have antibodies to nuclear components or phospholipids at the time of diagnosis, indicating that the development of autoantibodies precedes the onset of clinical symptoms [53]. 4-HNE modification of the SLE and Sjögren's syndrome antigen, Ro-60, results in autoimmunity and the development of a lupus-like phenotype in rodent models. The extent of the modification of the Ro-60 appears to dictate whether a SLE or Sjögren's syndrome phenotype is observed [11,54]. Interestingly, the genetic background of the mice, injected with Ro-60, significantly alters the observed phenotype [55]. This is an interesting observation in the context of the heterogeneity of SLE patients with similar autoantibody profiles.

Neo-epitope formation in non-nuclear antigens

The first component of complement, C1q, has been shown to participate in both the initiation of the complement cascade and in efferocytosis, two processes that are known to be important in SLE pathogenesis [46,56,57]. In addition, autoantibodies to C1q are strongly associated with the development of nephritis in SLE patients [58–60]. The oxidative modification of C1q is capable of increasing the antigenicity of the molecule and antibody titres towards oxidatively modified C1q, but not native C1q, correlate with renal disease severity in these patients [61–63].

Autoantibodies to MDA-modified epitopes are more prevalent in SLE patients than healthy control subjects These antibodies were determined to be predominantly of the IgM isotype when MDA-modified bovine serum albumin was used as the target antigen [64]. SLE patients have raised levels of IgG and IgM antibodies to MDA-modified low-density lipoprotein (LDL) compared to healthy subjects, whereas IgG anti-MDA-LDL levels were higher in SLE patients with cardiovascular disease than those without [65]. In addition, SLE patients demonstrate a higher level of oxidised LDL than healthy control subjects and both IgM and IgG reactivity correlated with disease activity [66]. These results suggest the systemic presence of IgM and IgG

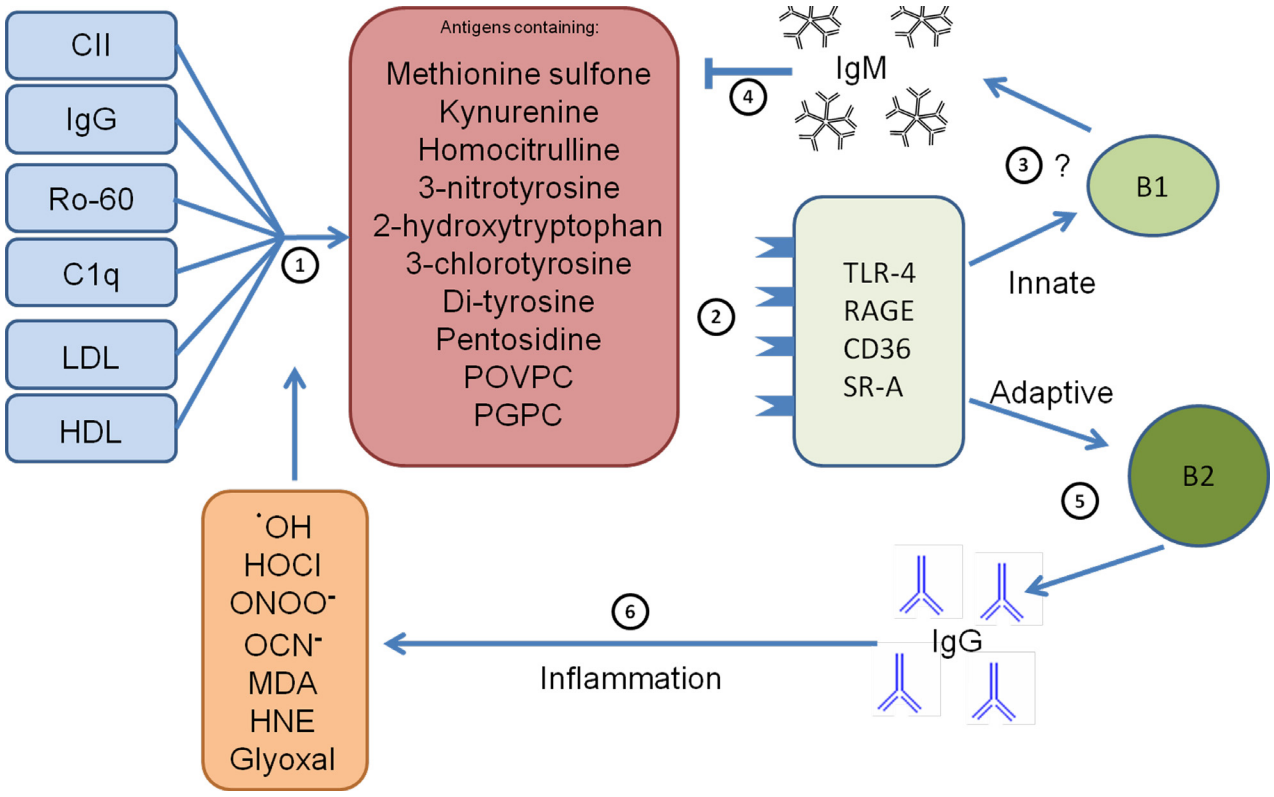

Fig. 3. Schematic representation of the role of oxidative PTMs in the initiation of autoimmunity. 1. Oxidants generated during inflammation modify amino acids in proteins known to be key antigens in inflammatory and autoimmune diseases. 2. These modified proteins or lipids represent neo-antigenic determinants that constitute neo-epitopes or pathogen/danger associated molecular patterns (PAMPs/DAMP)s and are recognised by the innate immune system via pattern recognition receptors (PRRs) such as toll-like receptors or scavenge receptors e.g. SR-A. 3. It appears that these neo-antigenic determinants result in an innate immune response, probably mediated by B1 cells resulting in production of IgM, which itself may act as a PRR. 4. IgM acts in a protective manner, possibly neutralising the 'neo-antigen'. 5. Neo-epitope formation aids in the breaking of tolerance and an adaptive (IgG) immune response ensues, followed by epitope spreading. 6. Production and binding of these autoantibodies to their antigens result in loss of protein function, cell/tissue damage and inflammation, which increases the production of free radicals and other reactive species. See text for references.

antibodies to MDA-modified autoantigens in SLE patients, and also suggest that MDA modification of LDL may be an important route of LDL modification in atherosclerosis. Indeed, expression of the natural antibody ppc1-5, which recognises both DNA and oxidised bovine serum albumin, elicited a protective effect in lupus prone mice (MRL-lpr), reducing immune complex deposition and proteinuria [67,68]. These benefits were associated with increased production of the anti-inflammatory cytokine, IL-10.

The data summarised above supports the hypothesis that oxidatively modified proteins elicit an innate (predominantly IgM) immune response. Upon prolonged exposure to this neo-antigen, an adaptive (IgG) response is mounted followed by epitope spreading to non-modified epitopes and eventually to other proteins complexed with the initial antigen (as summarised in Fig. 3).

Rheumatoid arthritis

Post-translational modifications involving citrullination and carbamylation

The most widely used clinical test for the diagnosis of RA is the measurement of anti-cyclic citrullinated peptide antibodies [20]. Some of the peptides, used in the original assay derived from proteins such as vimentin and fibrinogen, contain arginine residues. Arginine can undergo PTM to citrulline, catalysed by the enzyme peptidyl arginine deiminase (PAD) (Fig. 4a) [5,21,69]. In addition, carbamylation of proteins results in conversion of lysine to homocitrulline resulting in the loss of the positive charge of the lysine side chain, a process mediated by MPO catalysed cyanate formation (Fig. 4b and c). Carbamylation has been demonstrated to alter protein function, for example, LDL carbamylation influences inflammation via scavenger receptor A, albumin carbamylation inhibits a type-I collagen-induced respiratory burst, and MMP-9 activity can be altered by carbamylation of collagen (resulting in a modulation of extracellular matrix turnover) [70–72]. Given the structural similarity between citrulline (generated by PAD activity) and homocitrulline (a modification of lysine residues mediated by MPO catalysed cyanate formation), anti-citrullinated protein antibodies may also bind homocitrullinated antigens [73]. In addition, not all anti-carbamylated protein antibodies are expected to cross-react with citrullinated epitopes as antibody binding may be dependent on structural epitopes. Identification of cross-reactivity between the two has the potential for identifying subsets of RA patients [74].

Oxidative modification of rheumatoid factor

Additionally, many RA patients demonstrate the presence of rheumatoid factor (RF; antibodies targeting the Fc region of IgG) [20]. Although glycated IgG was detected in a number of inflammatory diseases, an increased proportion of RF-positive RA patients demonstrate antibodies to glycated IgG, compared to RF-negative patients [75]. In addition, IgG modified by a number of reactive oxygen species (ROS)-generating systems is a target for IgM isotype antibodies in RF-positive RA and SLE patients [76]. Oxidation of IgG impairs its function, altering both C1q and Fc receptor binding [77–79] Indeed, immunisation of rabbits with hydroxyl radical modified IgG induces a cross-reactive immune response mounted against IgG, nuclear components, as well as other antigens demonstrating that ROS may contribute to antibody cross-reactivity as observed in autoimmune diseases such as RA and SLE [80].

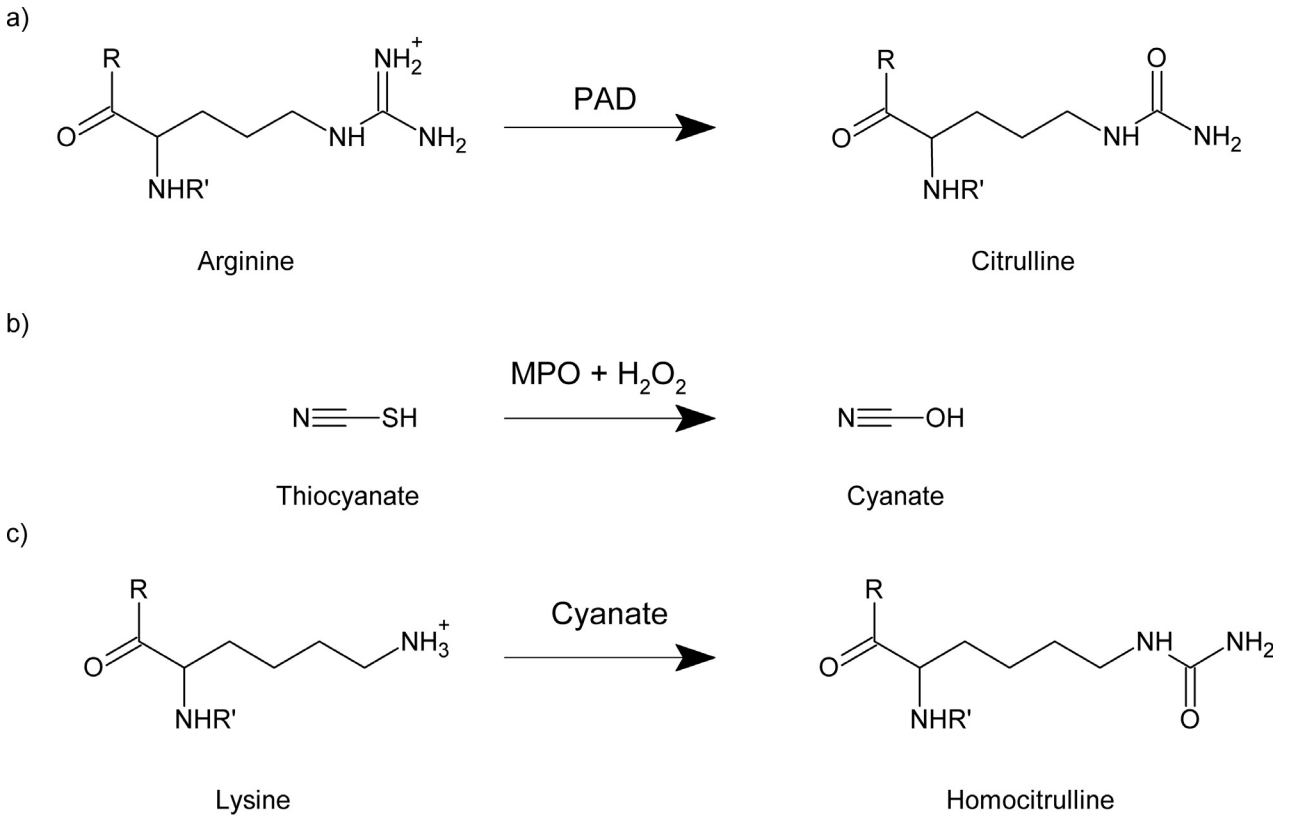

Fig. 4. Schematic representation of (a) citrullination of arginine by peptidyl arginine deiminase (PAD); (b) myeloperoxidase (MPO)-catalysed cyanate generation by oxidation of thiocyanate; and (c) conversion of lysine to homocitrulline by cyanate derived from the MPO-catalysed oxidation of thiocyanate to cyanate.

Autoantibodies to modified type-II collagen

Type-II collagen (CII) is one of the major autoantigens in the RA. We have demonstrated that modification of CII by oxidants including $^{\bullet}OH$, HOCl, $ONOO^{-}$ or glycation with ribose, increases the antigenicity of CII and results in aggregation and/or cleavage of the molecule [81]. In addition, antibodies to HOCl-modified CII are specific biomarkers of RA [82]. A greater proportion of early RA patients exhibited antibodies to HOCl-modified CII (90%) compared to unmodified CII (18%). Furthermore, the number of patients with anti-modified CII antibodies was higher in patients who did not respond to disease-modifying anti-rheumatic drugs (DMARDs; e.g. methotrexate) compared to patients who responded to DMARDs [82].

Type 1 diabetes

Serum autoantibodies in patients with autoimmune, type I, diabetes recognise $H_2O_2/CuCl_2$ induced aggregates of glutamic acid decarboxylase (GAD) [83]. Recently, we [84] demonstrated that oxidative modification significantly increased the antigenicity of CII in type-I diabetes patients that carried the HLA-DRB1*4 allele associated with increased RA prevalence, but to a lesser extent in patients without the RA associated HLA-DRB1*4. These data indicate a role of oxidative PTMs in autoimmunity in type-I diabetes patients.

Other chronic diseases with an autoimmune component

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease in which oxidative stress and the production of lipid peroxidation products play a key pathogenic role (as reviewed [8]). Indeed, COPD patients produce IgG1 isotype autoantibodies to carbonylated proteins [85]. The titre correlates with disease severity as does inflammatory C3 deposition in the lung [86].

3-Nitrotyrosine formation has been found to be prevalent in a number of cancers. The presence of 3-nitrotyrosine acts as a marker of the ability of CD8 + cells to respond to therapy in prostate cancer [87,88]. Despite the absence of IgG autoantibodies in cancer patients, large numbers of unmutated IgM molecules can be detected, indicating a role of immune surveillance in cancer progression [89–91].

An increased risk of atherosclerosis has been identified in RA, SLE, scleroderma and T1DM patients [92–95]. Research into both classical autoimmune diseases and the innate/adaptive immune responses in atherosclerosis provides informative parallels. The formation of oxidised LDL (oxLDL) is a key process in the aetiology of atherosclerosis. Both IgG and IgM isotype antibodies targeting oxLDL have been extensively described in these patients, with IgM isotype antibodies providing protection from the disease and the presence of IgG isotype antibodies being associated with a poorer prognosis [96]. IgM antibodies from atherosclerosis patients such as T15/E06 target the oxidised lipid moieties of LDL such as the phosphatidylcholine oxidation products 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine (POVPC) and 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine (PGPC) (Fig. 5) [97–99]. Recently, it has been demonstrated that antibodies to oxidised high-density lipoprotein (HDL) also exist in atherosclerosis patients. The oxidation of HDL emanates from an MPO-halide dependent oxidation of a tryptophan residue, forming 2-hydroxytryptophan (Fig. 1c) [100]. Interestingly, these anti-HDL antibodies target the protein moiety of the lipoprotein, as opposed to the lipid moiety, demonstrating the ability of antibodies to target both protein and lipid modifications in structurally similar molecules.

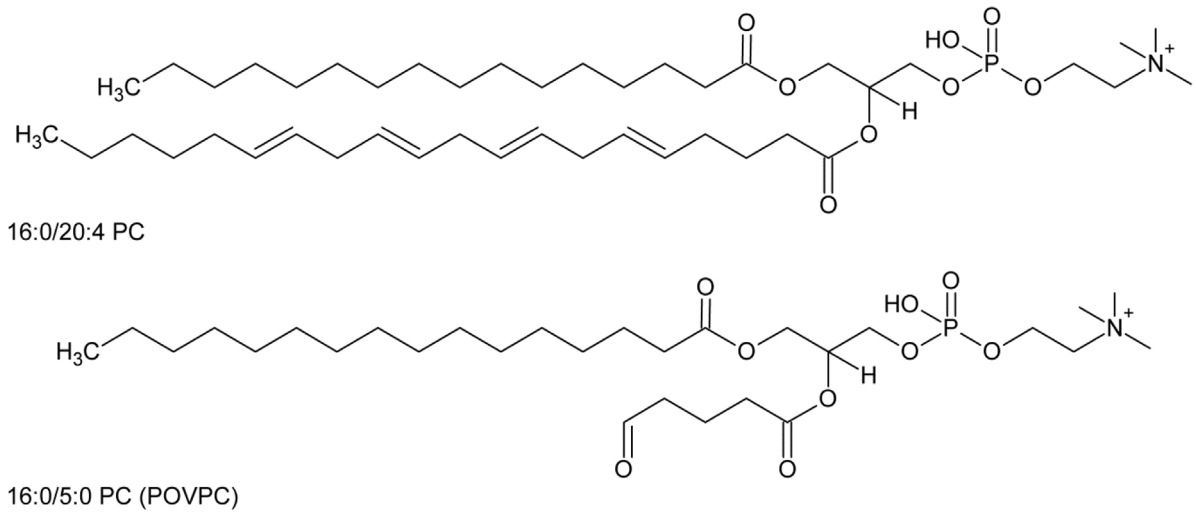

Fig. 5. Structures of (a) the unmodified phosphatidylcholine molecule (16:0/20:4 PC) and (b) the oxidised lipid product 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine (POVPC; 16:0/5:0 PC).

Future directions and challenges

Oxidative modifications as innate immune triggers

The oxidative PTM of host proteins is a frequent and necessary phenomenon required for processes such as cell signalling. However the nature of these modifications is usually reversible (e.g. cysteine sulfenic acid, S-nitrosothiol and methionine sulfoxide formation) and it may be conjectured that they probably do not usually initiate an immune response. In the case of stable PTMs, the chronic exposure to these post-translationally modified neo-epitopes/PAMPs may be enough to trigger the breakdown of tolerance in individuals with underlying impaired immune regulation. The observation that a large number of established innate PRRs, such as CD36, CRP and IgM, all recognise various oxidatively modified antigens indicates evolutionarily conserved mechanisms that are shared by both 'self' and 'foreign' PAMPs [101] (Fig. 3). The role of these innate immune sensors of neo-epitopes in autoimmunity remains to be elucidated, although the increasing number of examples of both innate and adaptive immune responses to neo-antigens indicates that these observations could translate into diagnostic and therapeutic benefits. However, both the identification and the proposed manipulation of the B1 cells thought to be responsible for this IgM secretion remain contentious issues [102].

Given the data available, it may be suggested that the key impairment in inflammatory autoimmune diseases is either the chronic production of neo-antigens or the dysregulation of the interface between 'protective' IgM responses and 'pathogenic' IgG responses. This leads to the hypothesis that in diseases such as RA and SLE (particularly patients with atherogenesis), increasing IgM titres are produced, over time, in response to sustained presentation of oxLDL to B cells. However, chronic presentation of oxLDL by antigen presenting cells (APCs) to B cells, or increasing amounts of oxLDL, in a pro-inflammatory environment, may initiate an IgG anti-oxLDL response. Interestingly, T15 antibodies responsible for binding to oxLDL are also able to recognise oxidised phospholipids expressed on the surface of apoptotic cells [97]. Given the well-established observations, both of defective apoptotic cell clearance in SLE patients and increased incidence of atherosclerosis in SLE patients (see discussion above), the potential for B1-cell-mediated natural antibody production in SLE warrants further investigation.

Clinical utility of responses against neo-epitopes

Modification of proteins by oxidants generates a wide variety of products, as described above. Whether there is a specific disease "fingerprint" of PTMs/sequences that is associated with modified antigenicity in particular diseases (possibly in combination with genetic background) remains to be investigated, although the solvent exposure of neo-epitopes likely plays a major role [55,103]. Aside from neo-epitope formation, oxidative modifications may affect the structure of a protein, resulting in unfolding [104]. This, in turn, exposes cryptic epitopes which are able to elicit an immune response [10]. Autoantibodies to oxidatively modified autoantigens have been identified in a number of autoimmune inflammatory diseases and there are increasing literature examples of the titres of these antibodies being biomarkers, as described above. Indeed, these neo-antigens have the potential to act as early and/or specific disease biomarkers. The demonstration that oxidative PTMs of major autoantigens in RA and SLE increases antigenicity has clear mechanistic implications for the aetiology of these diseases, but whether the presence of anti-oxidised protein antibodies is, in general, an epiphenomenon in the wider context of inflammatory disease remains to be elucidated.

A major challenge encountered in studies of neo-antigenicity is the identification of the peptide sequence/structural motifs containing the modified amino acid residue (antigenic determinant). A number of studies have reported interesting correlations between disease activity and antibody titres, using whole proteins modified with a promiscuous oxidant, as discussed above. The identities of the exact neo-epitope and antigenic determinants responsible for autoimmunity are difficult to determine, although progress will be possible using a combination of LC–MS/MS, molecular modelling and peptide-based ELISA approaches [26,27,62]. Once neo-epitopes have been identified, it is possible to develop diagnostic assays based only on the clinically-relevant epitope(s) – as opposed to whole proteins that may contain additional, confounding (non-clinically-relevant), epitopes. Assays based solely on clinically-relevant neo-epitopes are likely to exhibit higher clinical sensitivity and specificity, as exemplified in the development of the anti-CCP assay [105].

The heterogeneity of immune responses is a major factor in identifying immune responses in autoimmune diseases such as RA and SLE. For example antibodies to citrullinated antigens are an excellent diagnostic marker of RA, although not all RA patients are positive for anti-citrullinated peptide antibodies. Similarly, antibodies to PTM antigens may identify clinically-relevant sub-populations of patients such as patients who are likely not to respond to DMARDs, or biologics.

This principle is demonstrated by the case of HOCl-modified CII, discussed above [82]. Despite these potential advances, no single native or PTM antigen is likely to provide a definitively specific or sensitive clinical diagnosis or prognosis for polygenic conditions. Thus substantial clinical gains may be made by multiplexing assays – measuring immune responses against a number of native and PTM antigens – to develop more robust multi-biomarker diagnostic scores.

B-cell depletion therapy potentially offers a unique chance to identify the role of PTMs in the mechanism of autoantibody production, assuming the mechanism is identical in disease initiation and during B cell repopulation. For example, longitudinal samples could be taken from SLE patients who have received B cell depletion therapy and are in a subsequent phase of B cell repopulation. If anti-PTM antibody titres were observed to precede antibody production against the native antigen (as suggested by data using HNE-modified Ro-60 in SLE, see above) this would implicate PTMs in the breaking of tolerance to that antigen.

A step towards translating our understanding of PTM mechanisms to therapeutic applications is the identification and cloning of both human anti-oxLDL and anti-oxHDL antibodies using phage display libraries [100,101]. This approach has identified specific antibodies recognising PTM antigens by using phage-display in conjunction with PTM peptides as the target antigen for selection. This technique has been used to identify antibodies that recognise oxidatively modified antigens such as T15 and IK17, and subsequently a single chain Fv fragment that lacks immunostimulatory capacity but will 'neutralise' its oxidatively modified antigen [101,106], and suggests a framework for translating the observations of oxidised protein antibodies in autoimmune diseases into potential therapeutics. We have begun to take steps in this direction, in the context of RA, by generating a single-chain Fv fragment which specifically recognises oxidised CII [82]. Given the general lack of efficacy of clinically tested antioxidants in human autoimmune diseases [2], the inhibition of neo-epitope formation might be insufficient to modulate disease in patients with established disease, if we assume that the antioxidants used in clinical trials were able to inhibit neo-epitope formation at the tested doses. However, alternative therapeutic strategies involving the manipulation of the resulting immune responses – either targeting IgM production (potentially via B1 cells) or selectively targeting IgG responses (using neutralising peptides or single chain antibody fragments) – offer potential benefits as well as technical challenges.

Acknowledgements

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